39-Subg
Structural Basis of Gating by RCK Domains
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TrkH and its homologs TrkG and KtrB belong to a superfamily of K^+ transport proteins that are required for growth of bacteria in low external K^+ concentrations. The crystal structure of TrkH from *Vibrio parahaemolyticus* showed that TrkH forms a homodimer, and each protomer resembles a K^+ channel with a unique gating mechanism. TrkH assembles with TrkA, a soluble protein comprising two Regulate-Conductance-of-K^+, or RCK domains, which control the gating of certain K^+ channels. In K^+ channels, eight RCK domains form a four-fold symmetric gating ring that matches the four-fold symmetry of the channel. A dilation of the diameter of the gating ring directly translates into expansion of the pore-lining helices of the channel and hence opening of the permeation pathway. However, the gating ring expansion mechanism in K^+ channels appears incompatible with the dimeric architecture and the different gating mechanism in TrkH. In addition, although TrkH resembles a K^+ channel, its channel activity has never been demonstrated. We have recorded single-channel activity from TrkH in spheroplasts consistent with two partly coupled pores. We also found that channel activity is upregulated by ATP via TrkA. To understand how channel gating is regulated, we solved two structures of the TrkA tetrameric ring, one in complex with TrkH and one in isolation, in which the ring assumes two dramatically different conformations. The structures suggest a mechanism for how ATP increases the open probability of the TrkH ion channel by inducing conformational changes in TrkA.

40-Subg
Calcium Channel Regulation in the Fight-Or-Flight Response
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Voltage-gated calcium channel type 1.2 (Cav1.2) is activated in response to cardiac action potentials and conducts calcium entry that initiates excitation-contraction coupling. Cav1.2 activity is increased by stimulation of the beta-adrenergic receptor/cAMP-dependent protein kinase (PKA) signaling pathway in the fight-or-flight response. Regulation of Cav1.2 channels by PKA requires formation of a noncovalent autoinhibitory signaling complex consisting of the body of the Cav1.2 channel, its proteolytically processed distal C-terminal domain, an A Kinase Anchoring Protein (AKAP), and PKA. The distal C-terminus serves as an autoinhibitor of channel activity, and PKA phosphorylation of sites at the interface between the distal and proximal halves of the C-terminus relieves this autoinhibition in response to beta-adrenergic stimulation. Mice with gene deletions or mutations that prevent these regulatory events have impaired beta-adrenergic response, altered exercise behavior, and heart failure. Overall, our results give new insights into the molecular mechanisms that control calcium channel function and cardiovascular physiology in stress, exercise and heart failure.

41-Subg
The Open Conformation of a Voltage-Gated Sodium Channel Reveals the Transmembrane Pathway and Gating Mechanism
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In excitable cells, the initiation of an action potential results from the opening of voltage-gated sodium channels. In humans, mutations in different sodium channel isoforms have been shown to have causal relationships with neurological and cardiovascular diseases, and they therefore represent key targets for development of pharmaceutical drugs. Sodium channels are also present in some prokaryotes, where they appear to function in homeostasis, motility, and chemotaxis. All sodium channels undergo a series of conformational changes associated with their open, closed and inactivated functional states.
We have determined the crystal structure of the open conformation of the NavMs bacterial sodium channel pore (McCusker et al, 2012). It contrasts with the structure (Payandeh et al, 2011) of a closely-related bacterial channel, NavAb, which has a closed pore conformation. The differences, which in the open form produce an internal cavity accessible to the cytoplasmic surface, result from a bend/rotation about a central residue in the C-terminal transmembrane segment. The open activation gate is of sufficient diameter to allow passage of hydrated sodium ions. Comparisons between the forms suggest a mechanism of channel opening and closing which differs both from that of potassium channels, and from existing models for sodium channel opening/closing. Comparisons of the selectivity filters and fenestrations further suggest features important both for activity and for the design of new state-specific ligands/drugs.

42-Subg
A Regulatory Complex of the Mitochondrial Uniporter Ca^{2+} Channel
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Ca^{2+} flux across the mitochondrial inner membrane regulates bioenergetics, cytoplasmic Ca^{2+} signals and cell death pathways. Mitochondrial Ca^{2+} uptake occurs at regions of close apposition with intracellular Ca^{2+} release sites, driven by the inner membrane voltage generated by oxidative phosphorylation (OXPHOS) and mediated by a Ca^{2+} selective ion channel (MiCa) called the uniporter whose complete molecular identity remains unknown. Mitochondrial calcium uniporter (MCU) was identified as the likely ion-conducting pore. Mitochondrial matrix Ca^{2+} concentration is maintained 5-6 orders of magnitude lower than its thermodynamic equilibrium level, but the molecular mechanisms for how this is achieved are not clear. We found that MICU1, a mitochondrial protein previously suggested to be required for uniporter-mediated Ca^{2+} uptake, is instead required to preserve normal $[Ca^{2+}]_m$ under basal conditions. In its absence, mitochondria become constitutively loaded with Ca^{2+}, triggering excessive reactive oxygen species generation and sensitivity to apoptotic stress. MICU1 interacts with MCU and sets a Ca^{2+} threshold for $Ca^{2+}{}_m$ uptake without affecting kinetic properties of MCU-mediated Ca^{2+} uptake, a regulation that requires both functional Ca^{2+} binding EF hands in MICU1. Thus, MICU1 is a gatekeeper of MCU-mediated $Ca^{2+}{}_m$ uptake that is essential to prevent $[Ca^{2+}]_m$ overload and associated stress. Additionally, we have also identified CCDC90A, hereafter referred to as MCUR1 (Mitochondrial Calcium Uniporter Regulator 1), as an inner mitochondrial membrane protein required for MCU-dependent mitochondrial Ca^{2+} uptake. MCUR1 binds to MCU and regulates ruthenium red-sensitive MCU-dependent Ca^{2+} uptake. MCUR1 knockdown does not alter MCU localization, but abrogates Ca^{2+} uptake by energized mitochondria in intact and permeabilized cells. Ablation of MCUR1 disrupts OXPHOS, lowers cellular ATP, and activates AMP kinase-dependent pro-survival autophagy. Thus, MCUR1 and MICU1 are critical components of a mitochondrial uniporter channel complex required for mitochondrial Ca^{2+} uptake and maintenance of normal cellular bioenergetics.

43-Subg
Regulation of Voltage Sensor Movement in KCNQ Channels by KCNE Beta Subunits
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The KCNQ1 potassium channel plays widely different physiological roles different cell types. In the heart KCNQ1 forms part of the voltage-gated I_{Ks} channels that limit the duration of the cardiac action potential, whereas in epithelial cells KCNQ1 forms part of a voltage-independent K^+ channel that is important for ion secretion. The different functions of KCNQ1 are mainly due to the co-assembly of KCNQ1 with different KCNE beta subunits. For example, the I_{KS} channel consists of 4 α-subunits (KCNQ1) which assemble with 2 to 4 β subunits (KCNE1), whereas in epithelial cells KCNQ1 co-assembles with KCNE3 to form voltage-independent K^+ channels. Mutations in either KCNQ1 or KCNE1 cause cardiac arrhythmia syndromes. Here we use Voltage clamp fluorometry (VCF) to directly study the effects of wild type and mutant KCNE subunits on the voltage sensor movement in KCNQ1 channel. We assess the voltage sensor movement (fluorescence) and channel opening (current), in order to understand the coupling between the KCNQ1 voltage sensor and channel gate in the presence of KCNE subunits. Our data shows that KCNE1 splits the voltage sensor movement in two separates phases: one at hyperpolarized potentials that moves the voltage sensor to an active state and a second at more depolarized potentials, which is tightly coupled to channel opening. Interestingly, VCF shows that KCNE3 locks the voltage sensor of KCNQ1, presumably in its activated state, thereby generating a voltage-independent K^+ channel. Furthermore, arrhythmia-causing mutations in KCNE1 shift the voltage dependence of the two different voltage sensor movements, revealing some of the molecular mechanisms underlying these arrhythmia-causing mutations. Our data suggests a putative mechanism for how KCNE1 subunit exerts its effects on the voltage sensor movement during I_{Ks} channel activation.

Subgroup: Mechanobiology

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From Matrix Elasticity to Nuclear Physics in Lineage Programs
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